

AMENDMENTS TO THE CLAIMS:

This listing of claims will replace all prior versions, and listings, of claims in the application:

LISTING OF CLAIMS:

1-158 (canceled)

159. (new) A kit for directionally ligating a double-stranded nucleic acid to a first adaptor sequence, the kit comprising:

(A) a deoxynucleotidetriphosphate mixture comprising:

(1) (a) an unmodified dATP selected from the group consisting of dATP and analogs thereof, (b) an unmodified dGTP selected from the group consisting of dGTP and analogs thereof, (c) an unmodified dCTP selected from the group consisting of dCTP and analogs thereof, and (d) (i) an unmodified dTTP selected from the group consisting of dTTP and analogs thereof, or (ii) an unmodified dUTP selected from the group consisting of dUTP and analogs thereof, wherein the analogs of dATP, dGTP, dCTP, dTTP, and dUTP do not impart resistance against enzymatic degradation by an exonuclease relative to dATP, dGTP, dCTP, dTTP, and dUTP, respectively, when incorporated into a polynucleotide; and

(2) at least one modified deoxynucleotidetriphosphate selected from the group consisting of a modified dATP, a modified dGTP, a modified dCTP, a modified dTTP, and a modified dUTP wherein the modified dATP, modified dGTP, modified dCTP, modified dTTP, or modified dUTP when incorporated into a polynucleotide imparts resistance, relative to dATP, dGTP, dCTP, dTTP, and dUTP, respectively, against enzymatic degradation by an exonuclease at the site of incorporation; and

(B) instructions for using the deoxynucleotidetriphosphate mixture in a procedure for directionally ligating a nucleic acid into a first adaptor sequence, wherein the adaptor sequence is a duplex nucleotide sequence for cohesive ligation to an end of an exonuclease digested amplification product.

160. (new) The kit of claim 159 wherein the instructions describe a directional ligation procedure comprising:

(A) forming an amplification product from a double-stranded nucleic acid using a reaction mixture comprising (1) the deoxynucleotidetriphosphate mixture, (2) a polymerase, (3) a first primer complimentary to a first strand of the double-stranded nucleic acid, said first primer having a first terminus complimentary to a first ligation site sequence of the first adaptor sequence, and (4) a second primer complimentary to a second strand of the double-stranded nucleic acid, said second primer having a second terminus complimentary to a second ligation site sequence of a second adaptor sequence, wherein the first terminus of the first primer and the second terminus of the second primer are not identical;

(B) treating the amplification product with an exonuclease to form a digested amplicon having a first overhang sequence at a first termini and a second overhang sequence at a second termini, wherein the first and second termini of said digested amplicon terminate at the sites of incorporation of the modified deoxynucleotidetriphosphate(s); and

(C) ligating the first overhang sequence of the digested amplicon to the first ligation site sequence of the first adaptor sequence.

161. (new) The kit of claim 159 wherein

(A) the mixture comprises a first unmodified deoxynucleotidetriphosphate and modified deoxynucleotidetriphosphate pair, unmodified dNTP₁ and modified dNTP₁, respectively, selected from the group consisting of (1) the unmodified dATP and the modified dATP, (2) the unmodified dGTP and the modified dGTP, (3) the unmodified dCTP and the modified dCTP, (4) the unmodified dTTP and the modified dTTP, and (5) the unmodified dUTP and the modified UTP; and

(B) the ratio of the modified dNTP₁ to the unmodified dNTP₁ is less than 20.

162. (new) The kit of claim 161 wherein the ratio of the modified dNTP₁ to the unmodified dNTP₁ is less than 9.

163. (new) The kit of claim 162 wherein the ratio of the modified dNTP₁ to the unmodified dNTP₁ is less than 1.

164. (new) The kit of claim 161 wherein the modified dNTP₁ is an alpha phosphate substituted deoxynucleotidetriphosphate.

165. (new) The kit of claim 164 wherein the ratio of the modified dNTP₁ to the unmodified dNTP₁ is between about 0.05 and 20.

166. (new) The kit of claim 165 wherein the ratio of the modified dNTP₁ to the unmodified dNTP₁ is between about 0.05 and 10.

167. (new) The kit of claim 166 wherein the ratio of the modified dNTP₁ to the unmodified dNTP₁ is between 0.05 and 4.

168. (new) The kit of claim 161 wherein the modified dNTP₁ is an alpha thiophosphorano deoxynucleotidetriphosphate.

169. (new) The kit of claim 168 wherein the unmodified dNTP₁ is an unmodified dATP and the modified dNTP₁ is an alpha thiophosphorano dATP.

170. (new) The kit of claim 169 wherein the ratio of the modified dNTP₁ to the unmodified dNTP₁ is between 0.05 and 10.

171. (new) The kit of claim 170 wherein the ratio of the modified dNTP₁ to the unmodified dNTP₁ is between 1.0 and 9.

172. (new) The kit of claim 171 wherein the ratio of the modified dNTP₁ to the unmodified dNTP₁ is between 1.5 and 5.

173. (new) The kit of claim 168 wherein the unmodified dNTP₁ is an unmodified dGTP and the modified dNTP₁ is an alpha thiophosphorano dGTP.

174. (new) The kit of claim 173 wherein the ratio of the modified dNTP₁ to the unmodified dNTP₁ is between 0.05 and 10.

175. (new) The kit of claim 174 wherein the ratio of the modified dNTP₁ to the unmodified dNTP₁ is between 0.05 and 7.

176. (new) The kit of claim 175 wherein the ratio of the modified dNTP₁ to the unmodified dNTP₁ is between 1 and 3.

177. (new) The kit of claim 161 wherein the modified dNTP₁ is an alpha boranophosphorano deoxynucleotidetriphosphate.

178. (new) The kit of claim 177 wherein the unmodified dNTP₁ is an unmodified dATP and the modified dNTP₁ is an alpha boranophosphorano dATP.

179. (new) The kit of claim 178 wherein the ratio of the modified dNTP₁ to the unmodified dNTP₁ is between 0.05 and 10.

180. (new) The kit of claim 179 wherein the ratio of the modified dNTP₁ to the unmodified dNTP₁ is between 0.05 and 4.

181. (new) The kit of claim 178 wherein the ratio of the modified dNTP₁ to the unmodified dNTP₁ is between 0.01 and 2.

182. (new) The kit of claim 177 wherein the unmodified dNTP₁ is an unmodified dGTP and the modified dNTP₁ is an alpha boranophosphorano dGTP.

183. (new) The kit of claim 182 wherein the ratio of the modified dNTP₁ to the unmodified dNTP₁ is between 0.05 and 20.

184. (new) The kit of claim 183 wherein the ratio of the modified dNTP₁ to the unmodified dNTP₁ is between 0.1 and 10.

185. (new) The kit of claim 184 wherein the ratio of the modified dNTP₁ to the unmodified dNTP₁ is between 0.1 and 5.

186. (new) The kit of claim 159 wherein the mixture comprises:

(A) a first unmodified deoxynucleotidetriphosphate and modified deoxynucleotidetriphosphate pair, unmodified dNTP₁ and modified dNTP₁, respectively, selected from the group consisting of (1) the unmodified dATP and the modified dATP, (2) the unmodified dGTP and the modified dGTP, (3) the unmodified dCTP and modified dCTP, (4) unmodified dTTP and modified dTTP, and (5) unmodified dUTP and the modified UTP;

(B) a second unmodified deoxynucleotidetriphosphate and modified deoxynucleotidetriphosphate pair, unmodified dNTP₂ and modified dNTP₂, respectively, selected from the group consisting of (1) the unmodified dATP and the modified dATP, (2) the unmodified dGTP and the modified dGTP, (3) the unmodified dCTP and the modified dCTP, (4) the unmodified dTTP and the modified dTTP, and (5) the unmodified dUTP and the modified UTP, wherein said first and second pairs are different; and

(C) the ratio of the modified dNTP₁ to the modified dNTP₂ relative to the unmodified dNTP₁ to the unmodified dNTP₂ is less than 51.

187. (new) The kit of claim 186 wherein the ratio of the modified dNTP₁ to the modified dNTP₂ relative to the unmodified dNTP₁ to the unmodified dNTP₂ is less than 27.

188. (new) The kit of claim 187 wherein the ratio of the modified dNTP₁ to the modified dNTP₂ relative to the unmodified dNTP₁ to the unmodified dNTP₂ is less than 13.

189. (new) The kit of claim 186 wherein the modified dNTP₁ and the modified dNTP₂ are alpha phosphate substituted deoxynucleotidetriphosphates.

190. (new) The kit of claim 189 wherein the ratio of the modified dNTP₁ to the modified dNTP₂ relative to the unmodified dNTP₁ to the unmodified dNTP₂ is between about 0.05 and 6.4.

191. (new) The kit of claim 190 wherein the ratio of the modified dNTP₁ to the modified dNTP₂ relative to the unmodified dNTP₁ to the unmodified dNTP₂ is between about 0.1 and 3.2.

192. (new) The kit of claim 191 wherein the ratio of the modified dNTP₁ to the modified dNTP₂ relative to the unmodified dNTP₁ to the unmodified dNTP₂ is between about 0.2 and 1.6.

193. (new) The kit of claim 190 wherein the unmodified dNTP₁ is the unmodified dGTP, the unmodified dNTP₂ is the unmodified dATP, the modified dNTP₁ is an alpha thiophosphorano dGTP, and the modified dNTP₂ is an alpha thiophosphorano dATP.

194. (new) The kit of claim 186 wherein the modified dNTP₁ and the modified dNTP₂ are alpha thiophosphorano deoxynucleotidetriphosphates.

195. (new) The kit of claim 194 wherein the unmodified dNTP₁ is the unmodified dGTP, the unmodified dNTP₂ is the unmodified dATP, the modified dNTP₁ is an alpha thiophosphorano dGTP, and the modified dNTP₂ is an alpha thiophosphorano dATP.

196. (new) The kit of claim 195 wherein the ratio of the alpha thiophosphorano dGTP to the alpha thiophosphorano dATP relative to the unmodified dGTP to the unmodified dATP is between 0.8 and 5.3.

197. (new) The kit of claim 195 wherein the ratio of the alpha thiophosphorano dGTP to the alpha thiophosphorano dATP relative to the unmodified dGTP to the unmodified dATP is between 0.17 and 2.7.

198. (new) The kit of claim 197 wherein the ratio of the alpha thiophosphorano dGTP to the alpha thiophosphorano dATP relative to the unmodified dGTP to the unmodified dATP is between 0.33 and 1.33.

199. (new) The kit of claim 198 wherein the ratio of the alpha thiophosphorano dGTP to the alpha thiophosphorano dATP relative to the unmodified dGTP to the unmodified dATP is about 0.66.

200. (new) The kit of claim 186 wherein the modified dNTP₁ and the modified dNTP₂ are alpha boranophosphorano deoxynucleotidetriphosphates.

201. (new) The kit of claim 200 wherein the unmodified dNTP₁ is the unmodified dGTP, the unmodified dNTP₂ is the unmodified dATP, the modified dNTP₁ is an alpha boranophosphorano dGTP, and the modified dNTP₂ is an alpha boranophosphorano dATP.

202. (new) The kit of claim 201 wherein the ratio of the alpha boranophosphorano dGTP to the alpha boranophosphorano dATP relative to the unmodified dGTP to the unmodified dATP is between 0.05 and 6.4.

203. (new) The kit of claim 202 wherein the ratio of the alpha boranophosphorano dGTP to the alpha boranophosphorano dATP relative to the unmodified dGTP to the unmodified dATP is between 0.1 and 3.2.

204. (new) The kit of claim 203 wherein the ratio of the alpha boranophosphorano dGTP to the alpha boranophosphorano dATP relative to the unmodified dGTP to the unmodified dATP is between 0.2 and 1.6.

205. (new) The kit of claim 204 wherein the ratio of the alpha boranophosphorano dGTP to the alpha boranophosphorano dATP relative to the unmodified dGTP to the unmodified dATP is about 0.4.

206. (new) The kit of claim 159 further comprising a first adaptor sequence, wherein the first adaptor sequence comprises a nucleotide sequence encoding at least one epitope tag.

207. (new) The kit of claim 206 wherein the first adaptor sequence comprises an epitope tag selected from the group consisting of c-myc, polyhistidine, polyarginine, glutathione-S-transferase (GST) tag, HA epitope, V5, and DYKDDDDK.

208. (new) The kit of claim 207 wherein the first adaptor sequence comprises a DYKDDDDK epitope tag.

209. (new) The kit of claim 206 further comprising a second adaptor sequence.

210. (new) The kit of claim 209 wherein the second adaptor sequence comprises a nucleotide sequence encoding at least one epitope tag and the epitope tag comprises c-myc, polyhistidine, polyarginine, glutathione-S-transferase (GST) tag, HA epitope, V5, or sequence DYKDDDDK.

211. (new) The kit of claim 210 wherein at least one epitope tag of the second adaptor sequence comprises the sequence DYKDDDDK.

212. (new) The kit of claim 159 further comprising an exonuclease.

213. (new) The kit of claim 212 wherein the exonuclease is a 3' to 5' exonuclease.

214. (new) The kit of claim 213 wherein the exonuclease is exonuclease III.

215. (new) The kit of claim 159 further comprising a first primer complimentary to a first strand of the double-stranded nucleic acid, the first primer having a first terminus complimentary to a first ligation site sequence of the first adaptor sequence, and a second primer complimentary to a second strand of the double-stranded nucleic acid, the second primer having a second terminus complimentary to a second ligation site sequence of a second adaptor sequence, wherein the first terminus of the first primer and the second terminus of the second primer are not identical.

216. (new) The kit of claim 215 wherein the first terminus or the second terminus is about one to about ten nucleotides in length.

217. (new) The kit of claim 216 wherein the first terminus or the second terminus is two to seven nucleotides in length.

218. (new) The kit of claim 217 wherein the first terminus or the second terminus is two to five nucleotides in length.

219. (new) The kit of claim 218 wherein the first terminus or the second terminus is four nucleotides in length.

220. (new) The kit of claim 215 wherein the first terminus is a 3' terminus and the second terminus is a 3' terminus.

221. (new) The kit of claim 215 wherein the first terminus is a 5' terminus and the second terminus is a 5' terminus.

222. (new) The kit of claim 221 wherein the first terminus is four nucleotides in length, the second terminus is four nucleotides in length, and the first terminus and the second terminus are not identical.

223. (new) The kit of claim 159 further comprising at least one polymerase.

224. (new) The kit of claim 223 wherein at least one polymerase is a thermostable polymerase.

225. (new) The kit of claim 224 wherein at least one polymerase is a Taq polymerase or a recombinant Taq polymerase.

226. (new) The kit of claim 223 wherein there are at least two polymerases.

227. (new) The kit of claim 159 further comprising instructions for using the deoxynucleotidetriphosphate mixture in a procedure for directionally ligating the nucleic acid into a second adaptor sequence.

228. (new) The kit of claim 215 further comprising instructions for using the deoxynucleotidetriphosphate mixture in a procedure for directionally ligating the nucleic acid into a second adaptor sequence.

229. (new) A deoxynucleotidetriphosphate mixture comprising:

(A) (1) an unmodified dATP selected from the group consisting of dATP and analogs thereof, (2) an unmodified dGTP selected from the group consisting of dGTP and analogs thereof, (3) an unmodified dCTP selected from the group consisting of dCTP and analogs thereof, and (4) (a) an unmodified dTTP selected from the group consisting of dTTP and analogs thereof, or (b) an unmodified dUTP selected from the group consisting of dUTP and analogs thereof, wherein the analogs of dATP, dGTP, dCTP, dTTP, and dUTP do not impart resistance against enzymatic degradation by an

exonuclease relative to dATP, dGTP, dCTP, dTTP, and dUTP, respectively, when incorporated into a polynucleotide; and

(B) at least two modified deoxynucleotidetriphosphates selected from the group consisting of a modified dATP, a modified dGTP, a modified dCTP, a modified dTTP, and a modified dUTP wherein the modified dATP, modified dGTP, modified dCTP, modified dTTP, or modified dUTP when incorporated into a polynucleotide imparts resistance, relative to dATP, dGTP, dCTP, dTTP, and dUTP, respectively, against enzymatic degradation by an exonuclease at the site of incorporation.

230. (new) The dNTP mixture of claim 229 wherein the mixture comprises:

(A) a first unmodified deoxynucleotidetriphosphate and modified deoxynucleotidetriphosphate pair, unmodified dNTP₁ and modified dNTP₁, respectively, selected from the group consisting of (1) the unmodified dATP and the modified dATP, (2) the unmodified dGTP and the modified dGTP, (3) the unmodified dCTP and the modified dCTP, (4) the unmodified dTTP and the modified dTTP, and (5) the unmodified dUTP and the modified UTP; and

(B) a second unmodified deoxynucleotidetriphosphate and modified deoxynucleotidetriphosphate pair, unmodified dNTP₂ and modified dNTP₂, respectively, selected from the group consisting of (1) the unmodified dATP and the modified dATP, (2) the unmodified dGTP and the modified dGTP, (3) the unmodified dCTP and the modified dCTP, (4) the unmodified dTTP and the modified dTTP, and (5) the unmodified dUTP and the modified UTP, wherein said first and second pairs are different;

wherein the ratio of the modified dNTP₁ to the modified dNTP₂ relative to the unmodified dNTP₁ to the unmodified dNTP₂ is less than 51.

231. (new) The mixture of claim of 230 wherein the ratio of the modified dNTP₁ to the modified dNTP₂ relative to the unmodified dNTP₁ to the unmodified dNTP₂ is less than 27.

232. (new) The mixture of claim 231 wherein the ratio of the modified dNTP₁ to the modified dNTP₂ relative to the unmodified dNTP₁ to the unmodified dNTP₂ is less than 13.

233. (new) The mixture of claim 230 wherein the modified dNTP₁ and the modified dNTP₂ are alpha phosphate substituted deoxynucleotidetriphosphates.

234. (new) The mixture of claim 233 wherein the ratio of the alpha phosphate substituted dNTP₁ to the alpha phosphate substituted dNTP₂ relative to the unmodified dNTP₁ to the unmodified dNTP₂ is between 0.05 and 6.4.

235. (new) The mixture of claim 234 wherein the ratio of the alpha phosphate substituted dNTP₁ to the alpha phosphate substituted dNTP₂ relative to the unmodified dNTP₁ to the unmodified dNTP₂ is between 0.1 and 3.2.

236. (new) The mixture of claim 235 wherein the ratio of the alpha phosphate substituted dNTP₁ to the alpha phosphate substituted dNTP₂ relative to the unmodified dNTP₁ to the unmodified dNTP₂ is between 0.2 and 1.6.

237. (new) The mixture of claim 234 wherein the unmodified dNTP₁ is the unmodified dGTP, the unmodified dNTP₂ is the unmodified dATP, the modified dNTP₁ is an alpha thiophosphorano dGTP, and the modified dNTP₂ is an alpha thiophosphorano dATP.

238. (new) The mixture of claim 233 wherein the modified dNTP₁ and the modified dNTP₂ are alpha thiophosphorano deoxynucleotidetriphosphates.

239. (new) The mixture of claim 238 wherein the unmodified dNTP₁ is the unmodified dGTP, the unmodified dNTP₂ is the unmodified dATP, the modified dNTP₁ is an alpha thiophosphorano dGTP, and the modified dNTP₂ is an alpha thiophosphorano dATP.

240. (new) The mixture of claim 239 wherein the ratio of the alpha thiophosphorano dGTP to the alpha thiophosphorano dATP relative to the unmodified dGTP to the unmodified dATP is between 0.8 and 5.3.

241. (new) The mixture of claim 239 wherein the ratio of alpha thiophosphorano dGTP to the alpha thiophosphorano dATP relative to the unmodified dGTP to the unmodified dATP is between 0.17 and 2.7.

242. (new) The mixture of claim 241 wherein the ratio of the alpha thiophosphorano dGTP to the alpha thiophosphorano dATP relative to the unmodified dGTP to the unmodified dATP is between 0.33 and 1.33.

243. (new) The mixture of claim 242 wherein the ratio of the alpha thiophosphorano dGTP to the alpha thiophosphorano dATP relative to the unmodified dGTP to the unmodified dATP is about 0.66.

244. (new) The mixture of claim 233 wherein the modified dNTP₁ and the modified dNTP₂ are alpha boranophosphorano deoxynucleotidetriphosphates.

245. (new) The mixture of claim 244 wherein the unmodified dNTP₁ is the unmodified dGTP, the unmodified dNTP₂ is the unmodified dATP, the modified dNTP₁ is an alpha boranophosphorano dGTP, and the modified dNTP₂ is an alpha boranophosphorano dATP.

246. (new) The mixture of claim 245 wherein the ratio of the alpha boranophosphorano dGTP to the alpha boranophosphorano dATP relative to the unmodified dGTP to the unmodified dATP is between 0.05 and 6.4.

247. (new) The mixture of claim 246 wherein the ratio of the alpha boranophosphorano dGTP to the alpha boranophosphorano dATP relative to the unmodified dGTP to the unmodified dATP is between 0.1 and 3.2.

248. (new) The mixture of claim 247 wherein the ratio of the alpha boranophosphorano dGTP to the alpha boranophosphorano dATP relative to the unmodified dGTP to the unmodified dATP is between 0.2 to 1.6.

249. (new) The mixture of claim 248 wherein the ratio of the alpha boranophosphorano dGTP to the alpha boranophosphorano dATP relative to the unmodified dGTP to the unmodified dATP is about 0.4.

250. (new) A kit for directionally ligating a double-stranded nucleic acid to a first adaptor sequence, the kit comprising:

(A) a deoxynucleotidetriphosphate (dNTP) mixture, the dNTP mixture comprising modified dNTPs for at least one of the four nucleotide triphosphates comprising dATP, dGTP, dCTP, dTTP and analogs thereof, which, when incorporated into a polynucleotide, impart resistance against enzymatic degradation by an exonuclease at the site of incorporation of the modified dNTPs; and

(B) a first adaptor sequence, wherein the first adaptor sequence comprises a nucleotide sequence encoding at least one epitope tag; and

(C) instructions for using the deoxynucleotidetriphosphate mixture in a procedure for directionally ligating a nucleic acid into a first adaptor sequence.

251. (new) The kit of claim 250 wherein the first adaptor sequence comprises an epitope tag selected from the group consisting of c-myc, polyhistidine, polyarginine, glutathione-S-transferase (GST) tag, HA epitope, V5, and DYKDDDDK.

252. (new) The kit of claim 251 wherein the first adaptor sequence comprises a DYKDDDDK epitope tag.

253. (new) A kit for directionally ligating a double-stranded nucleic acid to a first adaptor sequence, the kit comprising:

(A) a deoxynucleotidetriphosphate mixture comprising:

(1) (a) dATP, (b) dGTP, (c) dCTP, and (d) dTTP; and

(2) at least one modified deoxynucleotidetriphosphate selected from the group consisting of a modified dATP, a modified dGTP, a modified dCTP, a modified dTTP, and a modified dUTP wherein the modified dATP, modified dGTP, modified dCTP, or modified dTTP when incorporated into a polynucleotide imparts resistance, relative to dATP, dGTP, dCTP, and dTTP, respectively, against enzymatic degradation by an exonuclease at the site of incorporation;

(B) a first primer complimentary to a first strand of the double-stranded nucleic acid, the first primer having a first terminus complimentary to a first ligation site sequence of the first adaptor sequence;

(C) a second primer complimentary to a second strand of the double-stranded nucleic acid, the second primer having a second terminus complimentary to a second ligation site sequence of a second adaptor sequence, wherein the first terminus of the first primer and the second terminus of the second primer are not identical;

(D) an exonuclease;

(E) at least one polymerase; and

(F) instructions for using the deoxynucleotidetriphosphate mixture in a procedure for directionally ligating a nucleic acid into a first adaptor sequence and a second adaptor sequence, wherein the adaptor sequences are duplex nucleotide sequences for cohesive ligation to an end of an exonuclease digested amplification product.

254. (new) The kit of claim 253 further comprising:

(G) the first adaptor sequence, wherein the first adaptor sequence comprises a DYKDDDDK epitope tag; and

(H) the second adaptor sequence.

255. (new) The kit of claim 254 wherein the modified deoxynucleotidetriphosphates consist of an alpha thiophosphorano dGTP and an alpha thiophosphorano dATP.

256. (new) The kit of claim 255 wherein the first primer has a 5' terminus and the second primer has a 5' terminus.

257. (new) The kit of claim 256 wherein the exonuclease is an exonuclease III.

258. (new) The kit of claim 257 wherein at least one polymerase is a Taq polymerase or a recombinant Taq polymerase.

259. (new) The kit of claim 258 wherein there are at least two polymerases.